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FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. 100564-00045
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		DATE: January 19, 2001
		U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) 09/743800

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/05145	20 July 1999	20 July 1998

TITLE OF INVENTION: NOVEL UROKINASE INHIBITORS

APPLICANT(S) FOR DO/EO/US: Olaf WILHELM, Viktor MAGDOLEN, Jörg STÜRZEBECHER, John FOEKENS, Verena LUTZ

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
(THE BASIC FILING FEE IS ATTACHED)

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English [35 U.S.C. 371(c)(2)].

7. ☐ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].

9. ☐ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 - 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: PCT/IPEA/416, PCT/IPEA/409, PCT/ISA/210
CHECK NO. 309288
Drawings (6 sheets)

U.S. APPLN. NO. (IF KNOWN SEE 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO. PCT/EP99/05145	ATTORNEY DOCKET NO. 100564-00045
09/743800		DATE: January 19, 2001

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee [37 C.F.R. 1.492(a)(1)-(5)]:		
Search Report has been prepared by the EPO or JPO.....$860.00		
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482).....$690.00		
No international preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but international search fee paid to USPTO [37 C.F.R. 1.445(a)(2)].....$710.00		
Neither international preliminary examination fee (37 C.F.R. 1.482) or international search fee [37 C.F.R. 1.445(a)(2)] paid to USPTO.....$1,000.00		
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00		

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 860	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(e)].				$ 00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	18 - 20 =	00	X $ 18.00	$ 00	
Independent Claims	03 - 3 =	00	X $ 80.00	$ 00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$ 00	
TOTAL OF ABOVE CALCULATIONS =				$ 860	
Reduction by one-half for filing by small entity, if applicable. Applicant is entitled to Small Entity status. (Note 37 C.F.R. 1.9, 1.27, 1.28). -				$ 430	
SUBTOTAL =				$ 430	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(f)]. +				$ 00	
TOTAL NATIONAL FEE =				$ 430	
Fee for recording the enclosed assignment [37 C.F.R. 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). $40.00 per property +				$ 00	
TOTAL FEES ENCLOSED =				$ 430	
				Amount to be refunded	$
				Charged	$

a. ☒ A check in the amount of $430 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 01-2300 in the amount of $ to cover the above fee. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive [37 C.F.R. 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

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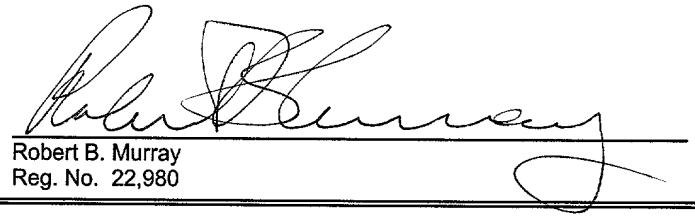

Robert B. Murray
Reg. No. 22,980

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Olaf WILHELM et al

Application No.: Unknown

Filed: January 19, 2001 Attorney Dkt. No.: 100564-00045

For: NOVEL UROKINASE INHIBITORS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231 January 19, 2001

Sir:

Prior to calculation of the filing fee and prior to the examination of this application, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 3, line 1, delete "or 2".

Claim 4, line 1, delete "any of claims 1 to 3" and insert therefor --claim 1--.

Claim 5, line 1, delete "any of claims 1 to 4" and insert therefor --claim 1--.

Claim 7, line 1, delete "any of claims 1 to 4" and insert therefor --claim 1--.

Claim 8, line 1, delete "any of claims 1 to 7" and insert therefor --claim 1--.

Claim 12, line 1, delete "or 11".

Claim 13, line 1, delete "any of claims 10 to 12" and insert therefor --claim 10--.

Claim 14, line 1, delete "any of claims 1 to 13" and insert therefor --claim 1--.

Claim 15, line 1, delete "any of claims 1 to 14" and insert therefor --claim 1--.

Claim 16, lines 4 and 5, delete "any of claims 1 to 14" and insert therefor --claim 1--.

Claim 17, line 4, delete "or 11".

REMARKS

The above amendment to the claims has been made to correct the multiple dependency of the claims and to put the application in better condition for examination.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,

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Novel urokinase inhibitors

Description

The invention relates to the use of derivatives of 3-amidinophenylalanine as urokinase inhibitors in particular for treating malignant tumors and the formation of metastases or as agents for targeting lymphocytes and for treating disorders of the lymphatic tissue, in particular lymphomas.

The ability of solid tumors to spread and metastasize in surrounding tissue correlates with the degradation or transformation of the extracellular matrix (tumor stroma) in the vicinity of the tumor cell and/or with the ability of said tumors to penetrate the basement membrane. Although the (patho)biochemical connections have not been completely elucidated yet, the plasminogen activator urokinase (uPA) and the urokinase receptor (uPAR) play a central role. uPA mediates the proteolytic cleavage of plasminogen to give plasmin. Plasmin in turn is a protease which has a wide range of actions and is capable of directly breaking down components of the extracellular matrix such as fibrin, fibronectin, laminin and the protein skeleton of proteoglycans. In addition, plasmin can activate "latent" metalloproteases and the inactive proenzyme of uPA, pro-uPA.

Tumor cells and non-malignant cells of the tumor stroma synthesize and secrete the enzymatically inactive proenzyme pro-uPA. Proteases such as, for example, plasmin or the cathepsins B and L cleave pro-uPA by limited proteolysis to give the active serine protease HMW-uPA (HMW = high molecular weight). Pro-uPA and the active protease HMW-uPA bind to the cell surface receptor uPAR (CD87). Plasmin(ogen) likewise binds to specific receptors on the plasma membranes of tumor

cells which leads to focused and amplified plasminogen activation in the immediate vicinity of the tumor cells. Invasive cells thus are able to break down the extracellular matrix without finding themselves deprived of the support necessary for directed movement because of proteolysis.

Various cytobiological studies have shown that the cell-associated plasminogen activator system is of particular importance within the cascade-like reaction pathways of tumor-associated proteolytic systems (Wilhelm et al. (1994 The Urokinase/Urokinase receptor system: A new target for cancer therapy? In: Schmitt M., Graeff H., Kindermann G. (eds.): Prospects in Diagnosis and Treatment of Cancer. International Congress Series, Excerpta Medica 1050, Amsterdam, Elsevier 1994, pp 145-156). Cultures of human colon carcinoma cells showed that their ability to migrate through an extracellular matrix depended on the degree of uPA receptor saturation with active uPA. (Hollas et al., Cancer Res. 51 (1991), 3690-3695). The cell culture model likewise showed a reduction in the invasive potential of cells when PAI-1 (Cajot et al., Proc. Natl. Acad. Sci. USA 87 (1990), 6939-6943) or PAI-2 (Baker et al., Cancer Res. 50 (1990), 4676-4684) inhibited the proteolytic activity of uPA. A similar effect was achieved on inhibition of uPA binding to the cell surface by blocking the receptor by means of proteolytically inactive uPA variants (Cohen et al., Blood 78 (1991), 479-487; Kobayashi et al., Br. J. Cancer 67 (1993), 537-544). Transfection of epidermoid carcinoma cells using a plasmid expressing an antisense transcript of a part of uPAR also reduced the invasivity of said cells by suppressing uPAR synthesis (Kook, EMBO J. 13 (1994), 3983-3991). Antibodies directed against uPA and PAI-1 reduced the invasive potential of lung cancer cells in vitro (Liu et al., Int. J. Cancer 60 (1995), 501-506).

Animal models of tumors were also able to show the influence of the plasminogen activator system on the metastasizing process. Thus, addition of anti-uPA antibodies almost completely prevented the formation of lung metastases caused by human carcinoma cells in chicken embryos (Ossowski and Reich, Cell 35 (1983), 611-619). Metastasizing human carcinoma cells were transfected using an expression plasmid which encoded a proteolytically inactive, but uPAR-binding uPA mutant. The mouse model showed that carcinoma cells synthesizing inactive uPA produced a significantly smaller number of metastases after injection than nontransfected cells (Crowley et al., Proc. Natl. Acad. Sci. USA 90 (1993), 5021-5025). Moreover, after administration of uPA antisense oligonucleotides, nude mice showed inhibition of intraperitoneal spreading of human ovarian carcinoma cells (Wilhelm et al., Clin. Exp. Metast. 13 (1995), 296-302).

In recent years, the clinical relevance of factors of the plasminogen activator system (uPA, uPAR, PAI-1 and PA1-2) for the prognosis of patients having solid malignant tumors has been intensively studied. In these studies, the uPA antigen content in various tumors (e.g. breast, ovaries, stomach, lung, kidney) proved to be a strong prognostic factor both for the recurrence-free survival and for the mortality (see for example, Schmitt et al., J. Obstet. Gynaecol. 21 (1995), 151-165; Jaenicke et al., Breast Cancer Res. Treat. 24 (1993), 195-208; Kuhn et al., Gynecol. Oncol. 55 (1994), 401-409; Nekarda et al., Lancet 343 (1994), 117; Pedersen et al., Cancer Res. 54 (1994), 4671-4675). Likewise, increased concentrations of uPAR in lung cancer tissue (Pedersen et al., supra) and breast cancer tissue (Duggan et al., Int. J. Cancer 61 (1995), 597-600; Ronne et al., Breast Cancer Res. Treat. 33 (1995), 199-207) and also in the case of stomach cancer both in the tumor tissue itself (Heiss et al., J. Clin. Oncol. 13 (1995), 2084-2093) and in tumor cells

disseminated into bone marrow (Heiss et al., Nature Medicine 1 (1995), 1035-1039) correlate with a poor prognosis.

The use of synthetic uPA inhibitors makes it possible to suppress invasion and spreading of tumor cells. However, developing specific uPA inhibitors is difficult, since tissue plasminogen activator (tPA) has an identical specificity for cleaving the peptide bond Arg560/Val561 of plasminogen. In most cases therefore, low molecular weight uPA inhibitors also inhibit tPA and thus also tPA-mediated fibrinolysis. In addition, it must be guaranteed that synthetic uPA inhibitors show no strong plasmin inhibition.

Despite these restrictions, some inhibitors are known which have a certain specificity for uPA, but a low inhibition capacity, such as benzamidine derivatives and β-naphthamidine derivatives, the most effective compound inhibiting uPA with K_i = 2.2 µmol/l (Stürzebecher and Markwardt, Pharmazie 33 (1978), 599), or amiloride with K_i = 7 µmol/l (Vassalli and Belin, FEBS. Lett. 214 (1987), 187-191).

DE-A-30 35 086 discloses cyclohexanecarboxylic acid derivatives which have inhibitory effects on proteases such as trypsin, chymotrypsin, thrombin or uPA. However, the compounds studied only show quite weak and, moreover, unspecific uPA inhibition. EP-A-0 183 271 discloses lysine derivatives and the use thereof as protease inhibitors. A benzamidinolysine derivative (compound 108) is also described which inhibits uPA in vitro, but acts comparably on other proteases such as trypsin or plasma kallikrein. WO 95/17885 disloses low molecular weight polypeptides as uPA inhibitors.

Another class of known uPA inhibitors is represented by 4-substituted benzothiophene-2-carboxamidines with K_i = 0.16 mmol/l in the case of benzothiophene 623 (Towle et

al., Cancer Res. 53 (1993), 2553-2559). These inhibitors have a significantly higher affinity for uPA than for tPA and plasmin. uPAR-bound uPA, too, is inhibited very effectively. Disadvantageously however, the chemical synthesis of these substances is complicated and few possibilities for structural modifications are present or have been demonstrated until now.

Therefore, the development of further uPA inhibitors is very beneficial for further elucidating the role of uPA and uPAR in various diseases, especially in tumor spreading and metastasizing.

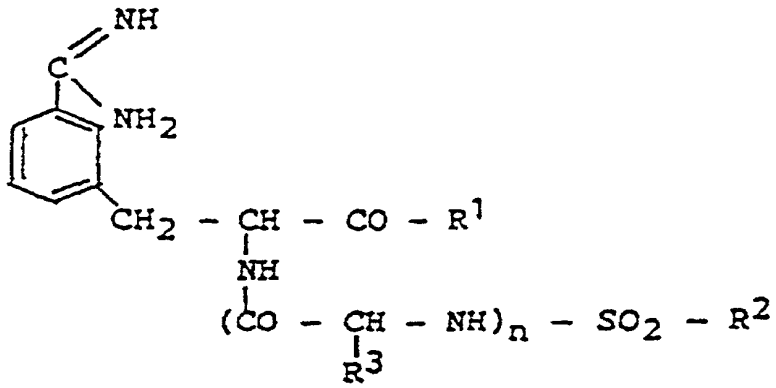
Nα-Arylsulfonyl and Nα-arylsulfonylaminoacyl derivatives of 3-amidinophenylalanine are known as selective inhibitors of thrombin (Markwardt et al., Thromb. Res. 17 (1980), 425-431) or of coagulation factor Xa (Stürzebecher et al., Thromb. Res. 54 (1989), 245-252). WO 92/08709, WO 94/18185 and WO 96/05189 also disclose the use of amidinophenylalanine derivatives as inhibitors of blood clotting, in particular as inhibitors of thrombin.

Piperidides and piperazides of 3-amidinophenylalanine have been intensively studied, among which lead structures for inhibiting fibrinolytic enzymes have been found (Stürzebecher et al., J. Enzyme Inhibition 9, 87-99, 1995; Stürzebecher et al., J. Med. Chem. 40, 3091-3099, 1997). While Stürzebecher et al. (1995) merely describe inhibition of thrombin, factor Xa, plasmin and trypsin, Stürzebecher et al. (1997) also provide information about inhibiting uPA. Nα-2-Naphthylsulfonyl-, Nα-2-(2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl- and Nα-2-camphor-10-yl-sulfonyl-substituted 3-amidinophenylalaninepiperazides have a K_i for uPA of from 28 to 140 µmol/l, which is about three orders of magnitude higher than the inhibition constant for thrombin. Thus it was impossible to assume that 3-

amidinophenylalanine derivatives are suitable as urokinase inhibitors.

Surprisingly we have found, however, that 3-amidinophenylalanine derivatives substituted in the 2 position by a phenyl radical represent selective uPA inhibitors which are active in vivo. Furthermore, we have found that these substances have high selectivity for lymphatic tissue and thus are suitable as agents for targeting lymphocytes, for example for treating malignant disorders of the lymphatic tissue such as lymphomas.

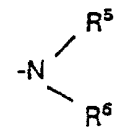
The present invention relates to novel urokinase inhibitors of the general formula I,



I

which are derived from 3-amidinophenylalanine and are present as racemates and also as L- or D-configured compounds and in which

R^1 (a) is OH or OR^4, where R^4 is unsubstituted or substituted, for example by hydroxyl, carboxyl, sulfonyl, nitro, cyano, oxo and/or halogen, branched or unbranched C_1-C_8-alkyl, C_3-C_8-cycloalkyl or aralkyl, e.g. benzyl or phenylethyl,

(b) represents a group of the formula  in

which R^5 and R^6 are any radicals compatible with the overall structure, where in particular

(i) R^5 and R^6 are H,

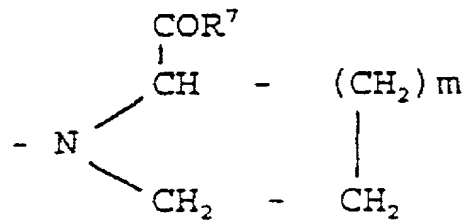
(ii) R^5 is H and R^6 is unsubstituted or substituted, for example by hydroxyl, carboxyl, sulfonyl, nitro, cyano, oxo and/or halogen, branched or unbranched C_1-C_8-alkyl, aralkyl, e.g. benzyl or phenylethyl, or C_5-C_8-cycloalkyl,

(iii) R^5 and R^6 are in each case independently unsubstituted or substituted, for example by hydroxyl or/and halogen, unbranched or branched C_1-C_4-alkyl or

(iv) R^5 is H and R^6 is $-NH_2$ or is, in particular, an aryl-substituted or heteroaryl-substituted amino group,

(v) R^5 is H or unsubstituted or substituted, for example by hydroxyl or/and halogen, unbranched or branched C_1-C_4-alkyl, and R^6 is an amino acid residue, for example an α-, β- or ω-amino carboxylic acid or amino sulfonic acid residue, a peptide residue, for example of up to 50 amino acids in length, or a polypeptide residue, for example of from greater than 50 amino acids to 1000 amino acids in length,

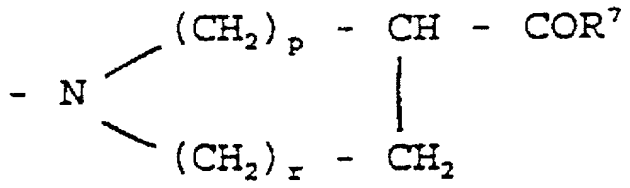
(c) represents a group of the formula



in which m is the number 1 or 2 and in which one or more of the methylene groups are optionally substituted, for example by

hydroxyl, carboxyl, C_1-C_4-alkyl or aralkyl, e.g. benzyl or phenylethyl, with the group (c) being racemic or in D or L configuration, and R^7 has the meaning of R^1 in subsections (a), (b) and (f),

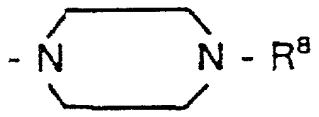
(d) represents a group of the formula



in which p = r = 1, p = 1 and r = 2 or p = 2 and r = 1 and in which one or more of the methylene groups are optionally substituted, for example by hydroxyl, carboxyl, C_1-C_4-alkyl or aralkyl, e.g. benzyl or phenylethyl, and R^7 has the meaning of R^1 in subsections (a), (b) and (f),

(e) represents a piperidyl group which is unsubstituted or substituted in one of positions 2, 3 or 4, for example by C_1-C_4-alkyl, C_1-C_3-alkoxy or hydroxyl,
where a further aromatic or cycloaliphatic ring, preferably phenyl or cyclohexyl, is optionally fused to the heterocycloaliphatic rings of the formulae (c), (d) and (e) in the 2,3 position or the 3,4 position relative to the heteroatom,

(f) represents a group of the formula



in which R^8 is

(i) unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-,

carboxyl, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted C_1-C_6-alkyl or aryl, such as, for example, phenyl, p-halophenyl or naphthyl,

(ii) saturated or unsaturated, branched or unbranched C_1-C_6-alkoxy or

(iii) unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted phenoxy or benzyloxycarbonyl,

(g) represents an acyl radical of the formula -COX, where X is

(i) H or unsubstituted, for example hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted, unbranched or branched alkyl, preferably C_1-C_6-alkyl, in particular methyl,

(ii) unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted aryl or heteroaryl, such as, for example, phenyl, p-halophenyl or thienyl, or

(iii) unsubstituted or, for example, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted cycloalkyl, preferably C_3-C_{10}-cycloalkyl,

(h) represents aralkyl, e.g. benzyl or phenylethyl, in which the aromatic radical is unsubstituted or substituted, for example by halogen, C_1-C_6-alkyl, C_1-C_3-alkoxy, hydroxyl, cyano, carboxyl, sulfonyl or nitro,

(i) represents a carboxamide radical of the formula -CONR'R" a thiocarboxamide radical -CSNR'R", or an acetamide radical -CH_2-CONR'R"

where

(i) R' and R" are H,

(ii) R' and R" are in each case independently C_1-C_4-alkyl,

(iii) R' is H and R" is C_1-C_4-alkyl,

(iv) R' is H and R" is aryl, e.g. phenyl, or

(v) R' and R" constitute together with the nitrogen atom a heterocycloaliphatic ring having 5-7 ring members and possibly having a further heteroatom, e.g. N, O or/and S,

(j) represents SO_2-Y where Y is

(i) unsubstituted or, for example, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted C_1-C_8-alkyl, preferably methyl, trifluoromethyl, trichloromethyl,

(ii) unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted aryl or heteroaryl, such as, for example, phenyl, 4-methylphenyl, 2,4,6-trimethylphenyl, 2,4,6-triisopropylphenyl, 4-methoxy-2,3,6-trimethylphenyl, 2,2-dimethyl-6-methoxy-chromanyl, 2,2,5,7,8-pentamethylchromanyl, anthraquinonyl, naphthyl or quinolyl, or O-aryl, preferably O-phenyl or O-heteroaryl or

(iii) -NR'R", where R' and R" are in each case independently H or C_1-C_3-alkyl,

(k) represents a cycloaliphatic ring having from 5 to 8 carbon atoms, which is unsubstituted or substituted, for example by C_1-C_6-alkyl, C_1-C_3-alkoxy, halogen, hydroxyl or/and oxo,

(l) represents an unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted heteroaryl radical such as, for example, pyridyl or pyrimidyl, or heterocycloaliphatic radical, for example N-methylpiperidyl,

(m) represents a functionalized alkyl radical of the formula $-(CH_2)_n-X$, where the alkyl chain is unbranched or branched, n = 1 to 8, and the functional radical X

(i) represents a hydroxyl group whose hydrogen atom is unsubstituted or substituted by C_1-C_4-alkyl, aralkyl, e.g. benzyl or phenylethyl, aryl, e.g. phenyl, C_1-C_4-hydroxyalkyl or acyl group CO-alkyl, (C_1-C_6),

(ii) is a halogen atom,

(iii) represents a tertiary amino group of the formula $-N(alk)_2$, where the alkyl groups have 1 to 3 carbon atoms and are preferably the same, and the nitrogen atom may belong to a heterocycloaliphatic ring having 5-7 ring members and possibly having a further heteroatom, e.g. N, O or/and S,

R^2 represents unsubstituted or, for example, C_1-C_6-alkyl-, C_1-C_3-alkoxy-, hydroxyl-, carboxyl-, sulfonyl-, nitro-, cyano-, oxo- or/and halogen-substituted phenyl, such as, for example, phenyl, 4-methylphenyl, 2,4,6-trimethylphenyl, 2,4,6-triisopropylphenyl, 4-methoxy-2,3,6-trimethylphenyl,

R^3 is H or branched or unbranched C_1-C_4-alkyl, and n is 0 or 1.

The compounds may also be present as salts, preferably as physiologically acceptable acid salts, for example as salts of mineral acids, particularly preferably as hydrochlorides, or as salts of suitable organic acids.

Of the compounds defined in the general claims, those are of particular importance in which R^1 corresponds to a group of the formulae (b), (d) and (f), R^2 represents phenyl mono-, di- or trisubstituted by alkyl, in particular 2,4,6-substituted phenyl, e.g. 2,4,6-triisopropylphenyl, and n = 0.

It is possible to prepare the compounds of the general formula I in a manner known in principle, for example as described in WO 92/08709 and WO 94/18185, and to assay their biological in vitro activity.

(L)-, (D) or (D,L)-3-cyanophenylalanine methyl ester hydrochloride is reacted with an appropriate sulfonyl chloride or a sulfonated amino acid or the halide thereof in the presence of a base to give a compound of the general formula I, which has a cyano function and in which $R^1 = OCH_3$ and R^2 and also R^3 and n correspond to the meanings defined in the general claims. Mild acidic or alkaline hydrolysis produces therefrom the compounds of the general formula I, which have carboxylic acid structure (R^1 = -OH) and whose acid-catalyzed esterification with an appropriate alcohol leads to compounds of the general formula I, where R^1 = (a). Applying a method common in peptide chemistry, for example DCC in the presence of HOBt, reacting the carboxylic acids of the general formula I (R^1 = OH) with a nucleophile of the structures (b), (e) and (f) may give compounds with the corresponding R^1 of the general formula I. To synthesize compounds with R^1 = (c) and (d), carboxylic acids of the general formula I with R^1 = OH are first reacted with cycloaliphatic amino acid esters of the structures (c) and (d), where R^7 is preferably $-OCH_3$ or OC_2H_5, the carboxylic esters obtained

are hydrolyzed under mild acidic or alkaline conditions to give the corresponding carboxylic acids which may subsequently be esterified in a manner already described or be reacted with nucleophiles of the structures (b), (e) and (f), and compounds of the general formula I with R^1 = (c) and also (d) and with R^7 = (a), (b), (e) and (f) are obtained.

The target compounds of the general formula I, which have amidine structure, are obtainable from the cyano compounds in a known manner; normally, the thioamides are obtained first by addition of H_2S to the cyano group, and are converted by S-methylation with methyl iodide into the thioimido esters and then into the amidino compounds by treatment with ammonium acetate in alcoholic solution. In addition and where appropriate, it is possible, using methanol or ethanol in the presence of HCl gas and, in particular cases, of an inert solvent, to prepare from the cyano compounds the corresponding imido ester hydrochlorides, which are reacted in alcoholic ammonia solution to give the amidino compounds.

The urokinase inhibitors according to the invention may be used, where appropriate, together with at least one suitable pharmaceutical excipient or carrier for producing orally, subcutaneously or intravenously administrable medicaments for controlling tumors or for diagnosis. Likewise possible is administration in combination with other active substances, for example other urokinase inhibitors such as antibodies or/and peptides.

The medicaments for controlling tumors in humans and animals may be administered topically, orally, rectally or parenterally, e.g. subcutaneously or intravenously, in the form of tablets, coated tablets, capsules, pellets, suppositories, solutions or transdermal systems such as plasters.

A particularly preferred compound of the formula (I) is Nα-(2,4,6-triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide hydrochloride or the L enantiomer thereof or a pharmaceutically suitable salt of these compounds. These substances have good solubility. They are soluble in Tris buffer (pH 7.3) up to a concentration of 5×10^{-5} mol/l. Addition of 5% ethanol increases the solubility to 2×10^{-4} mol/l and addition of 5% DMSO to 10^{-3} mol/l.

The compounds of the invention are capable of very effectively inhibiting the growth or/and spreading of malignant tumors, for example tumor spreading of pancreatic carcinoma, tumor growth of breast carcinoma and also metastasizing of tumors. It is possible to use the uPA inhibitors, where appropriate, together with other anti-tumor agents or with other types of treatment, e.g. radiation or surgery. Furthermore, the inhibitors according to the invention are also effective in other uPA-associated disorders (e.g. in preventing formation of blisters in the case of the skin disorder pemphigus vulgaris).

uPA inhibitors according to the invention are preferably characterized in that they have a K_i which is at least twofold, preferably at least 5-fold and particularly preferably at least 10-fold lower for uPA than for tPA. It is furthermore remarkable that the compounds of the invention only marginally affect blood clotting, since their K_i is too high for effective inhibition of thrombin and factor Xa.

The inventive substances of the formula I may be used in the form of conjugates with physiologically active substances, for example with radiolabels or cytotoxic agents, e.g. chemotherapeutics such as cisplatin or 5-fluorouracil, or with peptides. Furthermore it is possible to incorporate the substances into the

membrane of carrier vesicles, e.g. liposomes, and thus to facilitate targeting of active substances enclosed in the carrier vesicles, for example cytotoxic agents such as doxorubicin.

Another indication for the substances of the general formula II:

$$X\text{-}R^2,$$

where X is any radical, in particular an organic radical, for example a radical as defined for compounds of the formula I, but also another radical, for example a physiologically active substance such as a cytotoxic agent, a peptide or a radiolabel, a lipid or a carbohydrate, and R^2 is a group as defined above, in particular 2,4,6-trisubstituted phenyl, e.g. 2,4,6-triisopropylphenyl, is the targeting of lymphocytes, which is possible owing to a 10- to 20-fold higher affinity of said substances for lymph node tissue than for other types of tissue. R^2 is preferably linked to the radical X via an $-SO_2-$ sulfonyl group. Thus, these substances are excellently suited as diagnostic agents or as agents for treating diseases of the lymphatic tissue, in particular malignant diseases such as tumor metastases and lymphomas. Administering the substances may be carried out as already described above. Diseases of the lymphatic tissue are preferably treated by administering the medicament over a number of days, for example over a period of from 5 to 20 days, followed by a treatment break and, where appropriate, by one or more administration repeats.

The following examples and figures are intended to illustrate the invention in more detail. In the figures:

Figure 1 depicts the result from determining the cytotoxicity of a substance of the invention,

Figure 2 depicts the experimental result from inhibiting the degradation by human breast carcinoma cells of a fibrin matrix,

Figures 3 and 4 depict the effect of a substance of the invention on the spreading, growth and metastasizing of breast carcinoma cells in rats,

Figure 5 depicts the effect of a substance of the invention on the growth of a pancreatic tumor in rats, and

Figure 6 depicts the effect of a substance of the invention on the growth of human breast carcinoma cells in mice.

Examples

1. Nα-2,4,6-Triisopropylphenylsulfonyl-(L)-3-amidinophenylalanine 4-ethoxycarbonylpiperazide hydrochloride

1.1 Nα-2,4,6-Triisopropylphenylsulfonyl-(L)-3-cyanophenylalanine methyl ester

5 g of (L)-3-cyanophenylalanine methyl ester were suspended in 100 ml of dioxane, 4.45 ml of N-methylmorpholine (NMM) were added and the mixture was stirred for 30 min. After adding 5.97 g of 2,4,6-triisopropylbenzenesulfonyl chloride in solid form and subsequent stirring for 3 days, precipitated NMM-HCl was filtered off, the solvent was distilled off and the crude product obtained was purified on silica gel (SG) 60 (chloroform). Yield: 8.34 g of syrup (90%).

1.2 Nα-2,4,6-Triisopropylphenylsulfonyl-(L)-3-cyano-phenylalanine

8.34 g of compound 1.1 were heated under reflux in a mixture of 50 ml each of acetic acid and 1 N hydrochloric acid for 8 h and, after cooling, extracted twice with ethyl acetate; the combined ethyl acetate solutions were dried over $MgSO_4$ and the solvent was distilled off. After purification on SG 60 (chloroform), 5.8 g of a solid product were obtained (72%).

1.3 Nα-2,4,6-Triisopropylphenylsulfonyl-(L)-3-cyano-phenylalanine 4-ethoxycarbonylpiperazide

5.7 g of compound 1.2 were dissolved in 100 ml of tetrahydrofuran (THF) and cooled to 0°C; 2.22 g of α-hydroxybenzotriazole (HOBt) and 2.82 g of dicyclohexylcarbodiimide (DCC) were added and the mixture was stirred for 30 min. After adding 3.94 g of 1-ethoxycarbonylpiperazine in 30 ml of THF and subsequent stirring overnight, precipitated dicyclohexylurea (DCU) was filtered off, the solvent was distilled off and the crude product obtained was purified on SG 60 (chloroform). Yield: 7.1 g of an amorphous powder (96%).

1.4. Nα-2,4,6-Triisopropylphenylsulfonyl-(L)-3-amidino-phenylalanine 4-ethoxycarbonylpiperazide hydrochloride

7.1 g of compound 1.3 were dissolved in 30 ml of pyridine, 30 drops of triethanolamine (TEA) were added, a vigorous stream of hydrogen sulfide was introduced for 10 min, and the mixture was left at room temperature for 2 days. The solvent was then distilled off, the residue was dissolved in ethyl acetate, the organic phase was washed with 1 N

hydrochloric acid and saturated sodium chloride solution and dried over $MgSO_4$, and the solvent was distilled off. 7.2 g of thioamide obtained in this way were dissolved in 250 ml of acetone, 17 g of methyl iodide were added to the solution, and the mixture was left at room temperature under protection from light for 2 days. The solvent was then distilled off, the thioimido ester hydroiodide (8.5 g) was dissolved in 50 ml of methanol, 1.9 g of ammonium acetate were added and the mixture was heated to 60°C for 4 h. After distilling off the solvent, the crude product obtained was purified on Sephadex LH20 (methanol). The amidine hydroiodide obtained in this way was converted into the hydrochloride via an ion exchange column (Amberlite IRA-420). Yield: 5.3 g of an amorphous powder (69%).

2 Nα-2,4,6-triisopropylphenylsulfonyl-(D,L)-3-amidinophenylalanylnipecotic acid benzylamide hydrochloride

2.1 Ethyl Nα-2,4,6-triisopropylphenylsulfonyl-(D,L)-3-cyanophenylalanylnipecotate

4.56 g of Nα-2,4,6-triisopropylphenylsulfonyl-(D,L)-3-cyanophenylalanine (prepared from (D,L)-3-cyanophenylalanine methyl ester hydrochloride and the appropriate sulfonyl chloride analogously to 1.1 and 1.2), 1.5 g of HOBt and 2.42 g of DCC were dissolved in 50 ml of DMF; the mixture was stirred for 1 h, and then 2.36 g of ethyl nipecotate were added. After stirring overnight, precipitated DCU was filtered off, the solvent was distilled off and the residue was dissolved in a small amount of methanol and left to crystallize. The precipitate formed was filtered off with suction, washed with methanol and dried. Yield: 4.46 g (75%).

2.2 Nα-2,4,6-Triisopropylphenylsulfonyl-(D,L)-3-cyanophenylalanylnipecotic acid

4.4 g of the above-described ethyl ester were heated under reflux in a mixture of 35 ml of acetic acid and 25 ml of 1 N HCl for 2 h. After adding 10 ml of water, the mixture was left to cool, and a wax-like product precipitated. After decanting the solvent, 200 ml of water were added, the mixture was stirred over a relatively long period, and the solid substance obtained was filtered off with suction, washed with water and dried. Yield: 3.84 g (92%).

2.3 Nα-2,4,6-triisopropylphenylsulfonyl-(D,L)-3-cyanophenylalanylnipecotic acid benzylamide

2.28 g of the above-described compound, 0.6 g of HOBt and 0.97 g of DCC were dissolved in 20 ml of DMF and the mixture was stirred for 1 h; 0.6 g of benzylamine was then added, and stirring continued overnight. After filtering off the precipitated DCU, the solvent was distilled off, the residue was dissolved in methanol and the solution was poured into 5% strength sodium hydrogen carbonate solution/ice. After 1 h, the precipitate formed was filtered off with suction, washed with water and dried in vacuo. Yield: 2.48 g (94%).

2.4 Nα-2,4,6-triisopropylphenylsulfonyl-(D,L)-3-amidinophenylalanylnipecotic acid benzylamide hydrochloride

2.4 g of compound 2.3 were dissolved in 30 ml of pyridine, 30 drops of triethanolamine (TEA) were added, a vigorous stream of hydrogen sulfide was introduced for 10 min, and the mixture was left at room temperature for 2 days. The solvent was then distilled off, the residue was dissolved in ethyl

acetate, and extracted with 1 N hydrochloric acid. After washing the organic phase with saturated sodium chloride solution and drying over sodium sulfate, the solvent was distilled off. 2.38 g of the thioamide obtained in this way were dissolved in 100 ml of acetone, 6.5 g of methyl iodide were added to the solution, and the mixture was left at room temperature under protection from light for 20 h. The solvent was then distilled off, the thioimido ester hydroiodide was dissolved in 50 ml of methanol, 0.5 g of ammonium acetate was added and the mixture was heated to 60°C in a water bath for 4 h. After distilling off the solvent, the crude product obtained was purified on SG 60. Elution was carried out first with chloroform, then with chloroform/methanol 9:1. The amidine hydroiodide obtained in this way was converted into the hydrochloride on an ion exchange column (Amberlite IRA-420). Yield: 1.45 g of an amorphous powder (56%).

The compounds were characterized using mass spectrometry, and purity was checked by means of TLC and HPLC.

3. In vitro inhibition of urokinase by selected compounds of the formula I

Configuration	R^1	R^2	n	Ki, µmol/l
L	-N N-$COOC_2H_5$	TIPP	0	0.41
D,L	-N N-$COOC_2H_5$	TIPP	0	0.96

Abbreviations: TIPP - 2,4,6-triisopropylphenyl

Determination of inhibition activity

To determine the inhibitory activity, 200 µl of Tris buffer (0.05 mol/l, containing the inhibitor, 0.154 mol/l NaCl, 5% ethanol pH 8.0), 25 µl of substrate (Pefachrome UK or Bz-βAla-Gly-Arg-pNA in H_2O;

Pentapharm Ltd., Basle, Switzerland) and 50 µl of sc-urokinase (Ribosepharm GmbH, Haan, Germany) were incubated at 25°C. After 3 min the reaction was stopped by adding 25 µl of acetic acid (50%), and the absorption at 405 nm was determined by means of a Microplate Reader (MR 5000, Dynatech, Denkendorf, Germany). K_i values were determined according to Dixon by linear regression using a computer program. The K_i values are the mean values of at least three determinations with a standard deviation of less than 25%.

4. In vitro inhibition of various serine proteases of the trypsin type by (Nα-2,4,6-triisopropylphenylsulfonyl-(L)-3-amidinophenylalanine 4-ethoxycarbonylpiperazide (uPA inhibitor) compared with Nα-2-naphthylsulfonyl-3-amidinophenylalanine N'-methylpiperazide (naphthylsulfonyl derivative)

	Ki [µmol/l]	
Enzyme	**uPA-Inh.**	**Naphthylsulfonyl derivative**
Urokinase	0.41	150
Plasmin	0.39	55
Sc-tPA	4.9	430
Thrombin	0.49	0.036
Factor Xa	1.7	30
Factor XIIa	13	> 1000
Plasma kallikrein	7.2	85
Glandular kallikrein	> 1000	> 1000
Trypsin	0.037	1.3
Tryptase	6.3	33

The inhibition activities of the enzymes used were determined according to the principle described in Example 3.

The values given above indicate that the uPA inhibitor according to the invention has a K_i for urokinase which

according to the invention has a K_i for urokinase which is more than ten times smaller than the K_i for single chain tPA (Sc-tPA). Thus, the substances of the invention are suitable as selective urokinase inhibitors. For comparison, the inhibitory activity of the naphthylsulfonyl derivative is given which has a significantly lower in vitro anti-uPA activity.

5. Cytotoxicity determination

To determine cell proliferation/cytotoxicity a commercially available test was used (Promega) which is based on the cellular conversion of a tetrazolium salt. The colored product resulting from this reaction can be quantified by means of an ELISA spectrometer (ICN flow). The synthetic inhibitor (open circles) had no effect on the growth of the human ovarian carcinoma cells OV-MZ-6 (Figure 1) when compared with the solvent alone (closed circles). Thus, the inventive uPA inhibitor is not cytotoxic in pharmacologically effective concentrations up to 40 μM.

6. Inhibition of the degradation by human breast carcinoma cells of a fibrin matrix

To study the potential of tumor cells for breaking down an extracellular matrix, a fibrin matrix degradation assay was developed and used. A greater proteolytic activity of the tumor cells leads to a higher concentration of fibrin degradation products in the matrix supernatant. The matrix degradation capacity corresponds to the concentration of fibrin degradation products which are determined by means of ELISA (D dimer).

The fibrin gels were prepared in 24-well culture dishes from 200 ml of fibrinogen (50 mg/ml) in PBS (pH 7.4), by 50 μl of thrombin (10 U/ml) and 50 μl of $CaCl_2$ (150 mM) per well after incubation at 37°C for

30 minutes. 2×10^5 breast carcinoma cells were seeded on said fibrin matrix in 1 ml of DMEM culture medium plus 10% fetal calf serum and 2 µg of Glu-plasminogen, and incubated for 4 h. The supernatant was then centrifuged, in order to remove the cells, and the fibrin degradation products were quantified by means of ELISA. Adding the inhibitor (A) at different concentrations caused significant inhibition of matrix degradation by breast carcinoma cells compared with the naphthyl derivative (B) which shows no inhibition of fibrin degradation by breast carcinoma cells (Figure 2).

7. In vitro assay of the uPA inhibitor for tumor spreading, tumor growth and metastasizing in rats

A) Breast cancer model

10-25 mm^3 of BN-472 breast cancer tumor fragments from rats were transplanted into female brown Norwegian rats from 6 to 7 weeks old, subcutaneously and orthotopically under the subcutaneous fat of the mammary gland (Day 0). The treatment of the animals was started intraperitoneally 24 hours after tumor inoculation. Each group consisted of eight animals. The control group received only the injection solution (100 µl of a 10% ethanol/saline (0.9% NaCl) solution). A dose of 1 mg/kg body weight was intraperitoneally administered on a daily basis to the comparative group of the naphthyl derivative (B) and to the therapy group of the inventive uPA inhibitor (A). The treatment was carried out over a period of 4 weeks.

The dimensions of the subcutaneous tumors and the weight of the animals were determined weekly. At the end of the treatment the animals were sacrificed and tumor weights, organ weights and

the number of metastases in relevant tissues were determined.

Treatment with uPA inhibitor (A) resulted in a significant reduction in the weight of the primary tumor and also of the axillary lymph nodes ($p = 0.003$ and $p = 0.005$) compared with the naphthyl derivative (B) and control groups without inhibitor (Figs. 3 and 4). The weights of lung, liver, kidney and spleen were unchanged in the animals treated with the uPA inhibitor compared to the control animals.

B) Pancreatic carcinoma model

Fragments of the transplantable and metastasizing pancreatic adenocarcinoma CA20948 from rats were explanted from donor animals. After cell isolation, equal amounts of suspended tumor cells together with 2 mg of Matrigel were subcutaneously implanted into each of the acceptor animals, male 10 week-old Lewis rats (n=9). The treatment procedure and also the composition of the therapy groups were the same as under A).

Figure 5 shows for the inventive uPA inhibitor (open circles) a significant reduction in tumor weight and a decrease in the growth of developing rat pancreatic carcinomas compared to the naphthyl derivative (closed circles) and the control group (triangles).

C) Repeat of the experiments with different mode of administration

The experiments described in sections A and B were repeated with the inhibitor administered in a different way. For this, the inhibitor was administered subcutaneously in the breast

carcinoma model (n=9) and intraperitoneally in the pancreatic adenocarcinoma model (n=8) without changing the daily dose. The results of these repeat experiments corresponded to the results already discussed, both in tendency and extent.

D) Summary of the results

Treatment with the inhibitor achieved in all experiments a considerable reduction in tumor size and tumor weight and in the number and mass of metastases in comparison with the control groups. In the inhibitor-treated group of the breast tumor model, the average tumor weights at the end of the treatment were reduced to 23% (i.p.) or 37% (s.c.) compared to the vehicle-treated control. The number of lung foci in inhibitor-treated groups was reduced to 9% (i.p.) or 32% (s.c.) and the mean weights of the axillary lymph nodes to 27% (i.p.) or 48% (s.c.).

In the inhibitor-treated groups of the pancreatic tumor model, the mass of the tumor-containing pancreas was reduced by 76% (i.p.) or 34% (s.c.), and the masses of the subcutaneous tumors by 54% (i.p.) or 60% (s.c.), compared to the respective vehicle-treated groups. The number of detected liver foci in inhibitor-treated groups was 29% (i.p.), or 2% (s.c.), compared to the vehicle-treated control groups.

The development of the body weight increase and comparison of the organ weights between inhibitor-treated and vehicle-treated groups showed no indications of any considerable toxicity of the inhibitor under the described conditions.

8. Treatment of human breast cancer cells in nude mice

In order to test the in vivo efficacy of the inhibitor for inhibiting tumor growth of human breast carcinoma cells (MDA-BA-231), 6×10^6 cells were injected subcutaneously into the right flank of Balb/c nude mice (4-6 weeks old). The tumor cells were preincubated with the synthetic uPA inhibitor prior to inoculation. After 24 h, the mice were treated twice a week intraperitoneally with a dose of 1.2 mg/kg body weight as described under A). The tumor size was determined weekly by measuring the two largest diameters.

Figure 6 shows that the tumor volume increases significantly more slowly on administration of the uPA inhibitor (open circles) than in the control group (closed circles) in which ethanol in saline was administered.

9. Biodistribution of the inhibitor in rats

The biodistribution of the inhibitor was determined by two independent experiments in tissue extracts of rats which had been treated once a day with 1 mg/kg i.p. of inhibitor i.p. over a period of 5 or 10 days. For the lysis, in each case 100 mg of tissue were mechanically comminuted and mixed with 200 µl of 1% Triton X-100 in physiological NaCl solution. After adding 400 µl of ethanol, the mixture was sonicated for 1 min. The tissue extract was centrifuged at 12,000 × g for 15 min. For prepurification, the supernatant was applied to a C18 Silica Reversed Phase column (Sep-Pak® cartridge C18, 1 ml Water, Eschborn, Germany), equilibrated with 1 ml of methanol and 1 ml of water, washed successively with 2 × 1 ml H_2O, 1 ml 10% methanol, 1 ml H_2O, 1 ml 5% acetonitrile, 0.04% perchloric acid and 1 ml H_2O and eluted with 500 µl of 75% acetonitrile, 0.04% perchloric acid. HPLC analysis was carried out on a

reversed phase C18 silica column with a 5-55% strength acetonitrile gradient containing 0.04% perchloric acid.

The concentrations of the inhibitor and, for comparison, of a corresponding naphthylsulfonyl derivative in each of the tissue types studied (µg/g) and also in blood plasma and bile (µg/ml) are depicted in the following table.

Table

Distribution profile of a substance of the invention in various tissues and in blood plasma and bile in comparison with a naphthylsulfonyl derivative (Nα-2-naphthylsulfonyl-3-amidinophenylalanine 4-ethoxycarbonylpiperazide

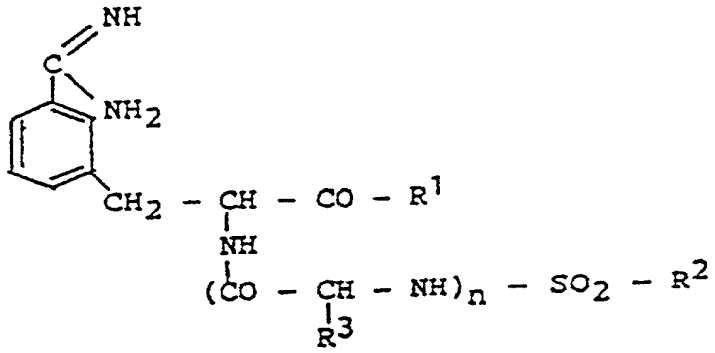
	Content (µg/g)		
Tissue	uPA inhibitor 1 mg/kg i.p. 5 days	uPA inhibitor 1 mg/kg i.p. 10 days	Naphthyl-sulfonyl derivative 1 mg/kg i.p. 5 days
Spleen	2.02	2.48	0.089
Liver	2.85	2.08	0.12
Kidney	2.67	2.48	0.085
Muscle	0.74	< 0.5	0.008
Fat/kidney	0.82	1.0	< 0.005
Heart	< 0.5	1.09	0.59
Lung	3.70	1.81	0.020
Brain	< 0.5	< 0.5	
Lymph nodes/trachea	7.45	16.38	0.12
lymph nodes/axillary	1.97	2.74	< 0.005
lymph nodes/knee	7.83	3.8	< 0.005
	Content (µg/ml)		
Plasma	0.008	0.035	0.004
Bile	1.96	1.75	0.097

In most of the tissue types studied, the inhibitor was present at from 1 to 3 µg/g after 5 to 10 days. 24 h after the last i.p. administration in each case, the plasma concentrations of the inhibitor were in each case one to two orders of magnitude below the mean tissue concentrations. From this, high tissue affinity and low plasma protein binding can be concluded. The concentrations of the naphthylsulfonyl derivative, administered for comparison over 5 days, were 20-30 times lower in the various tissues.

The inhibitor shows a noticeable accumulation in lymph nodes. In the independent experiments, concentrations of 5.3 and 7.5 µg/g, respectively, were measured in tracheal lymph nodes after 5 days of administration, and concentrations of 21.6 and 16.4 µg/g, respectively, after 10 days of administration. Since tumor cells often disseminate via lymph tracts, the specific accumulation of the inhibitor in lymphatic vessels is important and advantageous for its use as an anti-metastatic therapeutic agent.

Claims

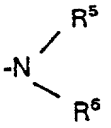
1. The use of compounds of the formula I



I

which are present as racemates and also as D- or L-configured compounds and in which

R^1 (a) is OH or OR^4, where R^4 is unsubstituted or substituted, branched or unbranched C_1-C_8-alkyl, C_3-C_8-cycloalkyl or aralkyl,

(b) represents a group of the formula 

in which R^5 and R^6 are any radicals, where in particular

(i) R^5 and R^6 are H,

(ii) R^5 is H and R^6 is unsubstituted or substituted, branched or unbranched C_1-C_8-alkyl, aralkyl or C_5-C_8-cycloalkyl,

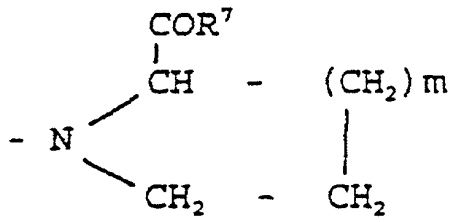
(iii) R^5 and R^6 are in each case independently unsubstituted or substituted, branched or unbranched C_1-C_4-alkyl or

(iv) R^5 is H and R^6 is $-NH_2$ or is, in particular, an aryl-substituted or heteroaryl-substituted amino group,

(v) R^5 is H or unsubstituted or

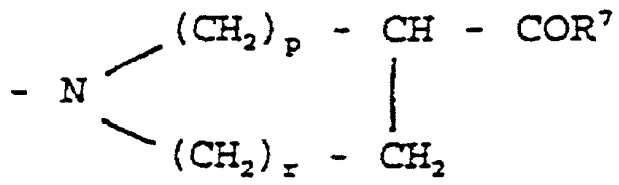
substituted, branched or unbranched C_1-C_4-alkyl or R^6 is an amino acid residue, a peptide residue or a polypeptide residue,

(c) represents a group of the formula



in which m is the number 1 or 2 and in which one or more of the methylene groups are unsubstituted or substituted, with the group (c) being racemic or in D or L configuration, and R^7 has the meaning of R^1 in subsections (a), (b) and (f),

(d) represents a group of the formula

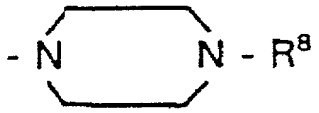


in which p = r = 1, p = 1 and r = 2 or p = 2 and r = 1 and in which one or more of the methylene groups are unsubstituted or substituted and R^7 has the meaning of R^1 in subsections (a), (b) and (f),

(e) represents a piperidyl group which is unsubstituted or substituted in one of positions 2, 3 or 4,
where a further aromatic or

cycloaliphatic ring is optionally fused to the heterocycloaliphatic rings of the formulae (c), (d) and (e) in the 2,3 position or the 3,4 position relative to the heteroatom,

(f) represents a group of the formula



in which R^8 is

(i) unsubstituted or substituted C_1-C_6-alkyl or aryl,

(ii) saturated or unsaturated, unbranched or branched C_1-C_6-alkoxy or

(iii) unsubstituted or substituted phenoxy or benzyloxycarbonyl,

(g) represents an acyl radical of the formula -COX, where X is

(i) H, unsubstituted or substituted, unbranched or branched alkyl

(ii) unsubstituted or substituted aryl or heteroaryl, or

(iii) unsubstituted or substituted cycloalkyl,

(h) represents aralkyl in which the aromatic radical is unsubstituted or substituted,

(i) represents a carboxamide radical of the formula -CONR'R", a thiocarboxamide radical, -CSNR'R" or an acetamide radical $-CH_2$-CONR'R" where

(i) R' and R" are H,

(ii) R' and R" are in each case independently C_1-C_4-alkyl,

(iii) R' is H and R" is C_1-C_4-alkyl,

(iv) R' is H and R" is aryl, or

(v) R' and R" constitute together with the nitrogen atom a heterocycloaliphatic ring having 5-7 ring members and possibly having a further heteroatom,

(j) represents SO_2-Y where Y is

(i) unsubstituted or substituted C_1-C_8-alkyl,

(ii) unsubstituted or substituted aryl or heteroaryl or O-aryl or O-heteroaryl or

(iii) -NR'R", where R' and R" are in each case independently H or C_1-C_3-alkyl,

(k) represents a cycloaliphatic unsubstituted or substituted ring having from 5 to 8 carbon atoms,

(l) represents an unsubstituted or substituted heteroaryl or heterocycloaliphatic radical,

(m) represents a functionalized alkyl radical of the formula $-(CH_2)_n$-X, where the alkyl chain is unbranched or branched, n = 1 to 8, and the functional radical X

(i) represents a hydroxyl group whose hydrogen atom is unsubstituted or substituted by C_1-C_4-alkyl-, aralkyl-, e.g. benzyl or phenylethyl, aryl, C_1-C_4-hydroxyalkyl or acyl group CO-alkyl (C_1-C_6),

(ii) is a halogen atom

(iii) represents a tertiary amino group of the formula $-N(alk)_2$, where the alkyl groups have 1 to 3 carbon atoms and the nitrogen atom may belong to a heterocycloaliphatic ring having 5-7 ring members and possibly having a further heteroatom, S,

R^2 represents unsubstituted or substituted phenyl,

R^3 is H or branched or unbranched C_1-C_4-alkyl, and n is 0 or 1,

or of salts of said compounds for preparing an agent for the diagnosis, therapy and prevention of urokinase-associated or urokinase receptor-associated disorders.

2. The use as claimed in claim 1, characterized in that R^1 is a group of the formulae (b), (d) and (f), R^2 represents 2,4,6 triisopropylphenyl, and n = 0.

3. The use as claimed in claim 1 or 2, characterized in that the compound of the formula I is Nα-(2,4,6-triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide, is the L enantiomer or a pharmaceutically suitable salt of one of the compounds.

4. The use as claimed in any of claims 1 to 3, characterized in that the compounds are present in the form of physiologically acceptable acid salts, in particular as hydrochlorides.

5. The use as claimed in any of claims 1 to 4 for controlling tumors.

6. The use as claimed in claim 5 for controlling breast carcinomas, pancreatic carcinomas and the formation of metastases.

7. The use as claimed in any of claims 1 to 4 for controlling pemphigus vulgaris.

8. The use as claimed in any of claims 1 to 7, characterized in that the compounds of the formula I are used coupled with further pharmacologically active substances.

9. The use as claimed in claim 8, characterized in that the compounds are used coupled with radiolabels or with cytotoxic substances.

10. The use of compounds of the formula II

$$X\text{-}R^2$$

where
X represents any radical and
R^2 represents unsubstituted or substituted phenyl, or of salts of said compounds for preparing an agent for targeting lymphocytes.

11. The use as claimed in claim 10, characterized in that R^2 is 2,4,6 trisubstituted phenyl, in particular 2,4,6 triisopropyl.

12. The use as claimed in claim 10 or 11 for diagnosing and treating disorders of the lymphatic tissue.

13. The use as claimed in any of claims 10 to 12 for controlling lymphomas and the formation of metastases.

14. The use as claimed in any of claims 1 to 13 for preparing medicaments which are administrable orally, topically, rectally or parenterally.

15. The use as claimed in any of claims 1 to 14 in the form of tablets, coated tablets, capsules, pellets, suppositories, solutions or transdermal systems such as plasters.

16. A method for inhibiting urokinase in living creatures, in particular in humans, by administering an effective quantity of at least one urokinase inhibitor as claimed in any of claims 1 to 4.

17. A method for targeting the lymphatic tissue in living creatures, in particular in humans, by administering an effective quantity of at least one compound as claimed in claim 10 or 11.

18. Nα(2,4,6-Triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide, the L enantiomer thereof or a pharmaceutically suitable salt of one of the compounds.

Abstract

The invention relates to the use of derivatives of 3-amidinophenylalanine as urokinase inhibitors for treating malignant tumors and the formation of metastases.

Fig. 1

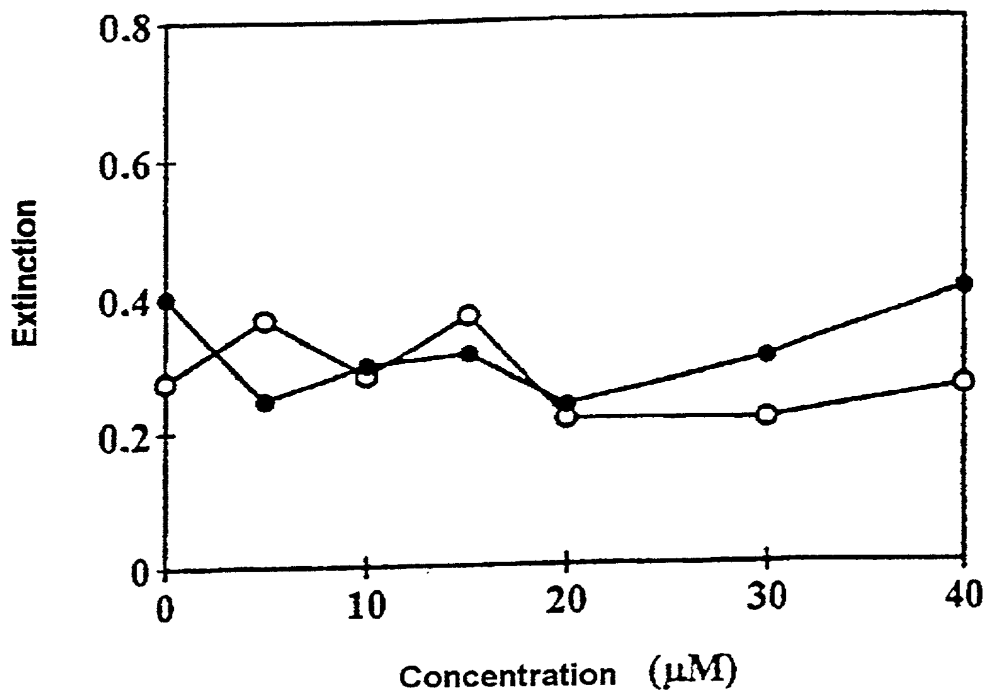


Fig. 2

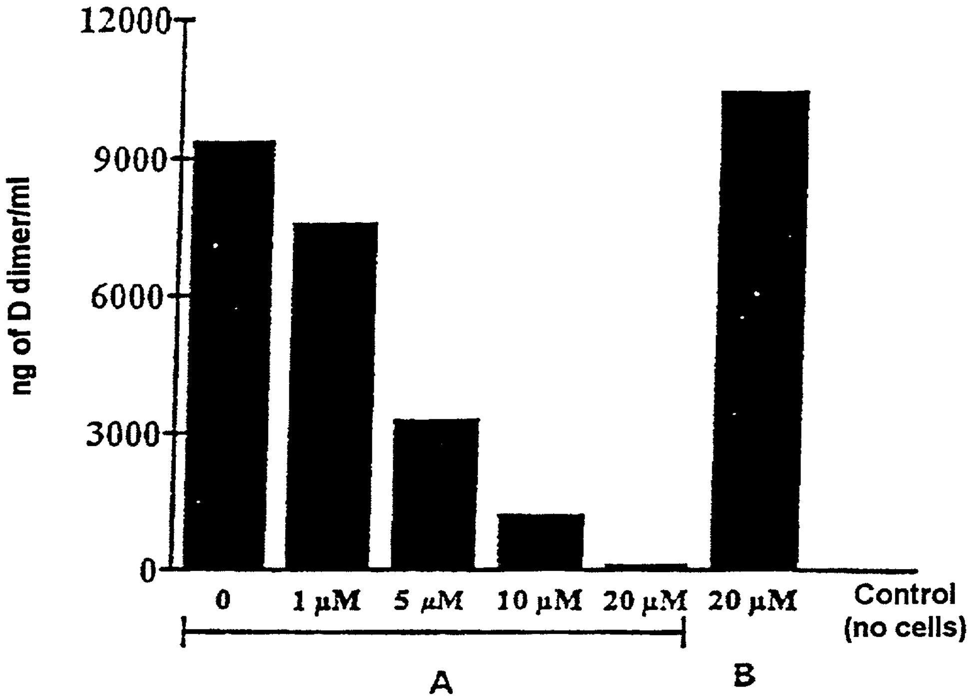


Fig. 3

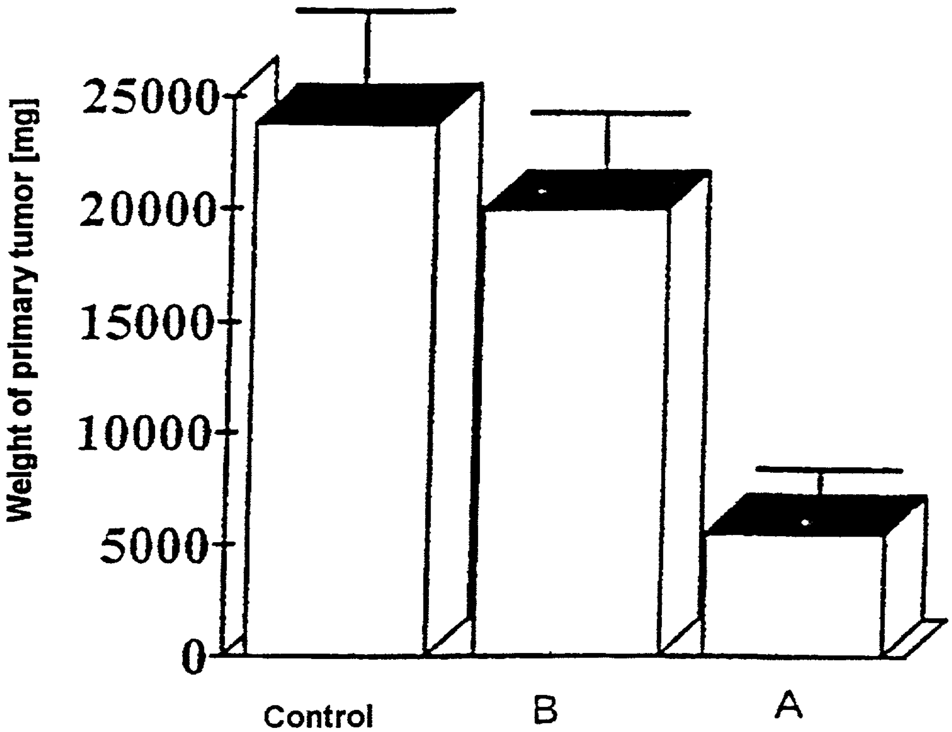

Weight of primary tumor [mg]
25000
20000
15000
10000
5000
0
Control
B
A

Fig. 4

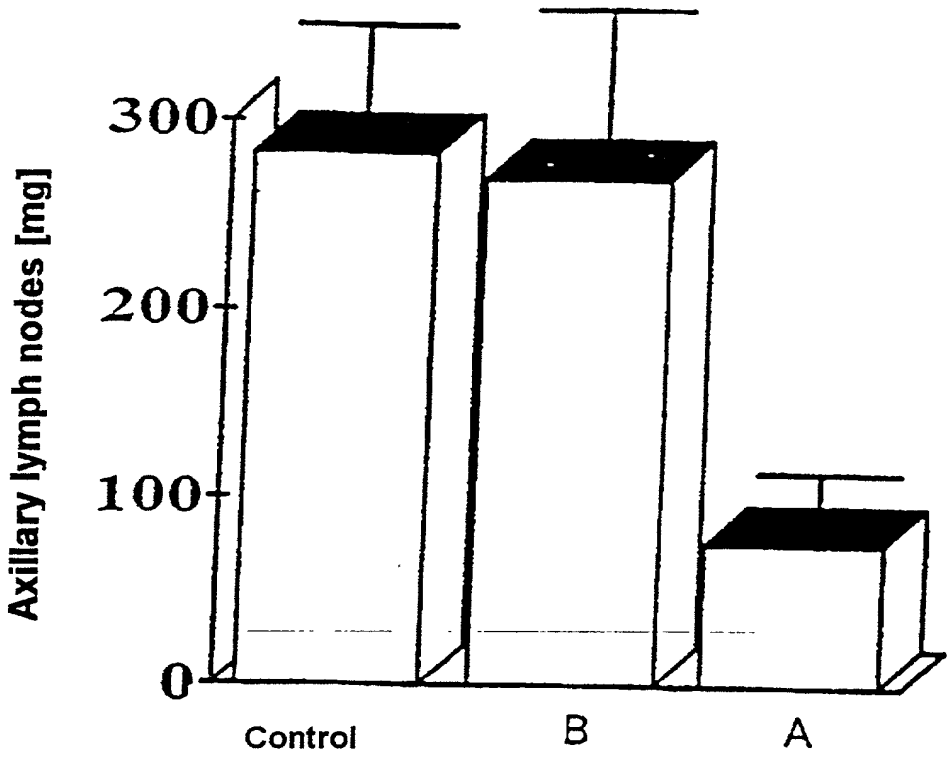


Fig. 5

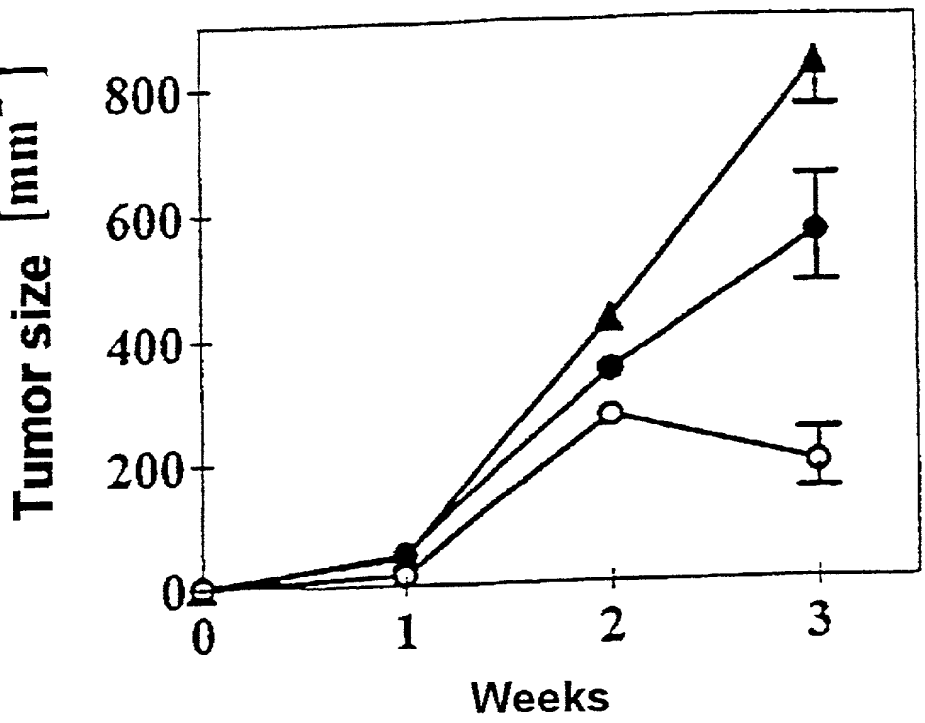
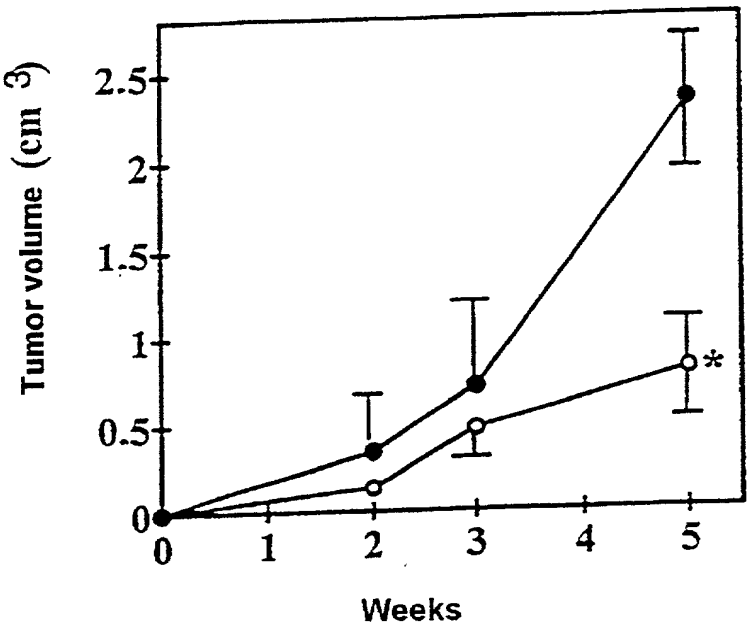


Fig. 6



* p = 0.014

Docket No. _____

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
Nikaido, Marmelstein, Murray & Oram Intellectual Property Group

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention **entitled**
(Insert Title) Novel Urokinase Inhibitors

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 20 July 1999 as PCT International Application
Number PCT/EP99/05145 and was amended on _____
and/or was filed on _____ as United States Application
Number _____ and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications. See note A on back of this page)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	98 113 519.7	Europe	20/July/1998	☒ Yes ☐ No
				☐ Yes ☐ No
				☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

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Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of eighth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of ninth joint inventor, if any _____

Inventor's signature _____ Date

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Post Office Address _____

Full name of eighth joint inventor, if any _____

Inventor's signature _____ Date

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Post Office Address _____

Full name of ninth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____